Combinatorial chemistry
Editorial overview

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Abbreviations
ADME absorption, distribution, metabolism and excretion
HTS high-throughput screeing

Combinatorial approaches increasingly impact virtually all areas of chemistry and biology. These approaches now play a central role in the study of the structure and function, as well as the engineering, of biopolymers, pervade all aspects of drug development and have increasingly begun to impact the fields of molecular recognition, catalysis and materials science. The inaugural issue of *Current Opinion in Chemical Biology* surveyed progress in the area of combinatorial chemistry. This issue of *Current Opinion in Chemical Biology* highlights advances that have been made over the past year in the development of increasingly sophisticated and multidisciplinary combinatorial tools, and in the knowledge that has been gained by implementing these tools. In addition, several areas are reviewed that were not covered in the inaugural issue.

In vitro selection of target-binding molecules and novel catalysts from oligonucleotide libraries provided a major focus for last year's overview of combinatorial research. Impressive progress continues to be made in this area; this is convincingly presented in the first article of this issue by Famulok and Jenne (pp 320–327). The spectrum of applications for which RNA or DNA aptamers are finding use ranges from elucidation of the biological function of nucleic acid binding proteins, to understanding molecular cues for RNA localization in selected subcellular compartments, and on to more biomedically oriented applications as both diagnostic markers for, and inhibitors of, inflammatory mediators *in vivo*. Clever selection strategies are also leading to the isolation of new ribozymes and deoxyribozymes capable of catalyzing an ever expanding list of chemical transformations.

Expression of combinatorially assembled oligonucleotides as peptide libraries in recombinant display vectors such as filamentous phage and related systems have been widely shown to provide powerful tools for peptide ligand discovery. Cell surface binding proteins for cytokines and growth factors constitute a large receptor class that includes a number of pharmaceutically important targets and which, historically, has proven refractory to the small-molecule screening efforts of many drug companies. Dower (pp 328–334) reviews the success of the Affymax group in using recombinant peptide libraries to identify high affinity antagonists and agonists for several such proteins, and these studies also serve to illuminate some important aspects of the nature of protein–protein binding interactions.

Combinatorial approaches to protein design face considerable challenges, in part because of the astronomical size of sequence space from which candidate molecules might be selected. There is currently significant interest in using evolutionary strategies to help solve biological design problems and, in particular, to exploit mutagenesis, recombination and selection in the evolution of biological function. Giver and Arnold (pp 335–338) highlight several strategies for *in vitro* DNA recombination (DNA shuffling) that have been used to enhance or otherwise alter the activity of single enzymes (and other proteins) as well as larger gene operons.

The power of biological combinatorial approaches is not limited to oligonucleotides, peptides and proteins. Considerable effort has also been directed towards harnessing the biosynthetic machinery responsible for the production of the vast array of small-molecule natural products found in nature. Staunton (pp 339–345) reviews the current status of the knowledge and tools that are being developed to enable the combinatorial biosynthesis of polyketide-based natural products. Combinatorial natural product biosynthesis could have immense practical utility due to the traditional and continued prominent role of natural product-based drugs.

Nowhere have combinatorial approaches weighed more heavily than in the field of drug discovery and development. The first area to be impacted was the synthesis and evaluation of small-molecule libraries for drug lead identification and optimization. Much of this effort is based upon the preparation of small-molecule libraries by solid phase synthesis or through the use of support-bound reagents or scavengers. The properties of the polymer support that is selected are essential to the success of these efforts. Labadie (pp 346–352) overviews the properties and applications of the most commonly used polymer supports and further surveys the recent development of supports designed to extend the range of chemistry available to the combinatorial chemist.

Two of the frontiers in small-molecule synthesis are the development of transition-metal-mediated reactions and

the development of domino reactions, which are highly efficient processes that allow the synthesis of complex molecules starting from simple substrates. Each of these reaction classes have attributes that are ideally suited for small-molecule library synthesis. Andres, Whitehouse and Deshpande (pp 353–362) describe the impressive array of transition-metal-mediated reactions that have been successfully employed in library generation. These reactions are particularly useful for library synthesis due to the generally mild reaction conditions and compatibility with a broad range of functional groups. Tietze and Lieb (pp 363–371) review the application of domino reactions in library synthesis that can provide access to large libraries of complex structures in relatively few steps from simple precursors. The power of domino reactions is most clearly demonstrated by the multicomponent condensation processes.

NMR methods have contributed more to the development of organic chemistry than any other analytical technique. Shapiro and Wareing (pp 372–375) describe the many important contributions that NMR methods have already had in combinatorial chemistry. In addition to the numerous NMR techniques that have been developed to characterize support-bound reaction intermediates and products, NMR is also increasingly being utilized for the high-throughput analysis of small-molecule libraries. Finally, NMR methods are not solely limited to compound characterization, as witnessed by powerful new approaches that have recently been developed to screen for compound–receptor interactions.

The goal of combinatorial chemistry is not to prepare and screen many molecules, but rather to identify molecules with sought-after properties or functions. How does one select the library of molecules to be prepared and screened out of the vast number of possibilities? Bures and Martin (pp 376–380) highlight computational methods that have been developed for the evaluation of compound similarity or dissimilarity and the application of these methods to the design of libraries and to the selection of compounds for high-throughput screening (HTS). Nature employs evolution and natural selection to optimize the properties of organisms without exploring all possibilities. Weber (pp 381–385) describes the successful applications of genetic algorithms to the identification of molecules with specific properties and functions whereby libraries of compounds are iteratively optimized through a computational process modeled on Darwinian evolution. As is true for traditional approaches to compound optimization, information about a library target can also greatly aid in selection of compounds for library preparation. Whittaker (pp 386–396) reviews the particularly successful application of this approach to the identification and optimization of potent inhibitors of proteases, where library design is guided by the mechanism or structure of the protease target.

HTS has become firmly established as a key activity in early-stage pharmaceutical discovery. Given the plethora of biological targets being identified through global efforts in gene sequencing, together with the potential compound windfall provided by combinatorial synthesis, it should be no surprise that there is intense interest in technologies that enhance the efficiency and productivity of HTS processes. Silverman, Campbell and Broach (pp 397–403) discuss recent advances in *in vitro* biochemical and cell-based assay technologies, with a particular focus on the use of homogeneous HTS methods and engineered recombinant cell lines for cellular reporter assays. The desire to achieve cost-effective and efficient screening of large compound collections is driving innovation towards higher density assay formats, and it seems likely that miniaturization technologies will continue to impact significantly on HTS methods over the next few years. One area in which assay miniaturization is already providing new tools is in genetic research, where densely ordered arrays of DNAs can be used in highly parallel hybridization assays. Southern and colleagues (pp 404–410) review methods for DNA array fabrication and the applications of these arrays in gene sequencing, polymorphism analysis and other gene sequence comparisons, as well as for gene expression monitoring.

Compounds selected for clinical development as human pharmaceuticals have key properties in addition to their potency and selectivity at some biological target site. Balancing compound potency with safety and appropriate pharmacokinetic profiles is mandatory in the identification of a marketable medicine. The vision of accelerated time-lines for drug development, driven by the output of combinatorial synthesis, may be undermined by the realization that *in vivo* assessment of pharmacokinetic properties of a lead candidate is already a key rate-limiting event in selecting molecules for development. Tarbit and Berman (pp 411–416) review efforts to apply higher throughput screening principles in order to evaluate the absorption, distribution, metabolism and excretion (ADME) properties of compounds, and to integrate such approaches earlier in the drug discovery process. Although this field is still at a very early stage of development, with considerable technical challenges to be faced, it seems clear that introduction of high-throughput ADME screens should ultimately lead to more efficient drug design.

The review by de Miguel and Sanders (pp 417–421) on the generation and screening of synthetic receptor libraries reminds us that nature does not have a lock on receptor design, and that chemists, too, can devise molecules capable of binding ligands with good selectivity and affinity. Selecting receptors with the desired recognition and/or catalytic functions from combinatorial libraries of host-like molecules is emerging as a particularly useful strategy. Sequence-selective peptide binding, enantioselective substrate recognition and selective transition-metal

ligation by members of metal ligand libraries are examples of some of the early success stories in combinatorial host–guest chemistry. Libraries of transition metal complexes are examined in greater detail in the final article by Francis, Jamison and Jacobsen (pp 422–428), with particular attention being made to the discovery of new catalysts from these libraries. The authors note that catalyst discovery poses considerable challenges from the perspective of rapid evaluation and screening methods, and it is clear that innovative new approaches will be necessary in order for combinatorial methods to have a major impact here. Combinatorial principles are also being applied to the preparation of multicomponent metal oxide materials by high temperature solid state synthesis methods. Parallel screens have been effectively applied for identification of materials with high T_C (critical temperature) superconductivity, giant magnetoresistance and useful phosphorescence properties.

The vibrant multidisciplinary nature of research in combinatorial technologies is well captured by the collection of articles in this issue of *Current Opinion in Chemical Biology*. In aggregate, the field can be seen as more than just a set of techniques for the generation and evaluation of molecules and materials, but rather as a research strategy for solving highly underdetermined problems. It is exciting to see these approaches begin to be embraced by disciplines as diverse as materials science and *in vivo* pharmacology, which have little or no tradition of HTS. The tangible impact of combinatorial methods in drug discovery will be closely scrutinized over the next one to two years and we can anticipate learning much more about successes and failures in integrating these new technologies into traditional industrial discovery activities. There is already a sense that high speed synthesis methods are proving valuable in compound optimization programs, where conventional medicinal chemistry receives a much-needed shot-in-the-arm through the power of parallel processing. A significantly greater uncertainty surrounds the use of library methods for *de novo* identification of high quality lead compounds, and it seems likely that winning strategies here will be particularly highly prized.